

One more *Microtus* species with asynaptic sex chromosomes

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Abstract: Arvicoline voles are known as a karyotypically extremely polymorphic group in which the genus *Microtus* leads with the highest rate of karyotype change. A member of this genus, the European pine vole *Microtus subterraneus* (de Selys-Longchamps, 1836), is widely distributed in Europe and parts of Asia. There are two cytotypes differing in diploid chromosome number, $2n=54$ and 52 , each showing different chromosomal polymorphisms. At two localities in southeastern Serbia, Mt. Jastrebac and Vlasina, we found the $2n=52$ cytotype. Meiotic preparations from males revealed the presence of asynaptic sex chromosomes. Although asynaptic sex chromosomes are frequent in *Microtus*, this is the first finding for *M. subterraneus*. From summarized data it appears that two-thirds of the studied species, mainly from *Microtus* and *Terricola* subgenera, possess asynaptic sex chromosomes.

Key words: *Microtus subterraneus*; asynaptic sex chromosomes; meiosis; pine vole; cytotypes

INTRODUCTION

It is estimated that from 166 [1] up to 180 million years ago [2], after divergence of the eutherian-marsupial ancestor from monotremes, the therian X and Y chromosomes evolved. Synapsis and recombination of sex chromosomes at prophase I are necessary to provide accurate disjunction at anaphase I, and their absence can lead to male sterility or reduced fertility [3,4]. Recombination of sex chromosomes in mammals is restricted to the pseudoautosomal region. The pairing region represents the saved homologous portion of the ancestral pair of autosomes [5]. This region enables synapsis and recombination of sex chromosomes during meiosis, although synapsis can be extended to other regions that are not homologous. Thus, synaptonemal complexes similar to those of autosomal bivalents have been observed in most examined mammalian sex chromosomes.

Nevertheless, in some mammalian species, synapsis between X and Y chromosomes is absent throughout meiotic prophase I. This situation is found in all species of marsupials [6], the sand rat, *Psammomys*

obesus [7], the southern pygmy mouse, *Baiomys musculus* [8], and many Arvicolinae species [9]. Arvicoline rodents are characterized by both synaptic and asynaptic sex chromosomes. Among mammals they figure as a karyotypically extremely polymorphic group in which the genus *Microtus* leads with the highest rate of changes [10], producing karyotypes ranging from 17 to 62 chromosomes [11,12].

A particularly striking polymorphism is linked with sex chromosomes, i.e. the way of determination, structure, configuration, pairing, behavior and evolution [13]. Megias-Nogales et al. [14] mentioned eleven species of the genus *Microtus* with asynaptic sex chromosomes, two *Terricola* (*Pitymys* in former reports) and one *Myopus* species. Species with synaptic sex chromosomes are also present in the genus *Microtus*. Asynaptic sex chromosomes have been found in most *Microtus* species of Palearctic phylogenetic lineage, while normal synapsis occurs in the species of Asian phylogenetic lineage [15].

The European pine vole, *Microtus subterraneus* (de Selys-Longchamps, 1836) is native to most of Eu-



Fig. 1. Karyotype of male *Microtus subterraneus*, $2n=52$ from the locality Vlasina (south-eastern Serbia).

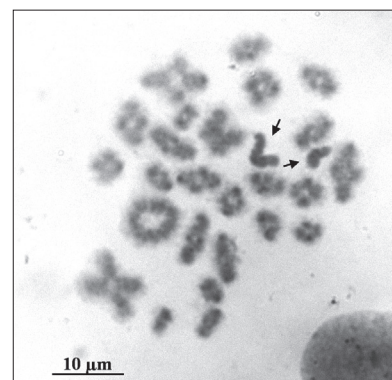


Fig. 2. C-banding of metaphase I of male meiosis *Microtus subterraneus* ($2n=52$), asynaptic sex chromosomes (indicated by arrows).

rope and parts of Asia [16]. In Europe, it is distributed from the Atlantic coast of France across central Europe up to the river Don in the east and the Balkans and northern Greece in the south. Isolated populations are also known from Estonia and northwestern Russia near St. Petersburg [17]. Furthermore, its presence is confirmed in Asia Minor [18,19]. Cytogenetic analysis revealed two cytotypes that differ in diploid chromosome number, $2n=54$ and 52 , with the same fundamental number ($FN=60$). This variation can be explained with a single Robertsonian fusion. Cytotype $2n=54$ is distributed in the northern part of central Europe, the European part of Russia and northern Anatolia, while animals with the $2n=52$ cytotype inhabit southern and eastern Europe. The karyotype form $2n=54$ is considered more conservative [20]. In addition, variations in size and morphology of X chromosomes have been reported between populations from Poland and Anatolia [19]. The second cytotype $2n=52$ showed variation in the size and morphology of the heterochromatic Y-chromosome [21,22] and in the morphology of the largest autosome pair [18,23,24]. Thus far there are no data on the type of sex chromosome pairing in this species.

MATERIALS AND METHODS

Sample collection

Animals were collected at two localities in south-eastern Serbia: Ravnište Mt. Jastrebac (43.429797 N, 21.370194 E) and Vlasina Rid (42.736060°N,

22.332332°E). Using Longworth traps, four males (ID: 771, 891, 900, 901) and two females (ID: 843, 920) were caught at the first locality, and two males (ID: 4117, 4220) and one female (ID: 4199) at the second. All animal procedures were approved by the Ethical Committee for the Use of Laboratory Animals of the Institute for Biological Research “Siniša Stanković”.

Chromosome preparations

Chromosome preparations were made directly from bone marrow and meiotic preparations from testis using standard techniques. Chromosomes were stained with 10% Giemsa solution. C-banding was performed with a slightly modified schedule according to Arrighi and Hsu [25]. At least ten meiotic configurations were analyzed per animal.

RESULTS

The obtained karyotypes of *M. subterraneus* in all studied animals had a diploid number of 52 (Fig. 1). The 52-chromosome karyotype consists of two pairs of large submetacentric autosomes, one pair of small metacentrics and 22 pairs of acrocentrics of decreasing size ($NF=60$). The X chromosome is a medium-sized metacentric and the Y chromosome is a medium-sized acrocentric.

Meiotic metaphase I configurations in all six analyzed males included 25 autosomal bivalents and two univalent sex chromosomes (Fig. 2). Both sex chro-

Table 1. List of *Microtus* species (assigned to corresponding subgenera) with synapctic/asynapctic sex chromosomes, diploid chromosome numbers (2n) and X/Y morphology.

Species	Subgenera [†]	2n	X/Y	References
Synapctic				
<i>M. fortis</i>	<i>Alexandromys</i>	52	M/A	[15]
<i>M. kikuchii</i>	<i>Alexandromys</i>	30	M/A	[31]
<i>M. mujanensis</i>	<i>Alexandromys</i>	38	SM/A	[15]
<i>M. maximowiczii</i>	<i>Alexandromys</i>	36-44	A/A	[15]
<i>M. montebelli</i>	<i>Alexandromys</i>	30	M/A	[32]
<i>M. oeconomus</i>	<i>Alexandromys</i>	30	M/A	[9,31]
<i>M. mandarinus</i>	<i>Lasiopodomys</i>	47-52	SA,M/A	[34,35]
<i>M. sikimensis</i>	<i>Neodon</i>	48	M/A	[33]
Asynapctic				
<i>M. agrestis</i>	<i>Agricola</i>	50	M/SA	[36,37]
<i>M. afghanus</i>	<i>Blanfordimys</i>	58	SA/A	[9]
<i>M. bucharicus</i>	<i>Blanfordimys</i>	48	A/A	[9]
<i>M. juldaschi</i>	<i>Blanfordimys</i>	54	M/A	[38]
<i>M. cabrerae</i>	<i>Iberomys</i>	54	M/SA,A	[39]
<i>M. brandtii</i>	<i>Lasiopodomys</i>	34	SA/A	[38]
<i>M. arvalis</i>	<i>Microtus</i>	46	M/M,A	[36,37]
<i>M. guentheri</i>	<i>Microtus</i>	52-54	A,SM/A,SM	[9]
<i>M. kirgisorum</i>	<i>Microtus</i>	54	SA/A	[9]
<i>M. rossiaemeridionalis</i>	<i>Microtus</i>	54	A/A	[9]
<i>M. socialis</i>	<i>Microtus</i>	62	A/A	[9]
<i>M. transcaspicus</i>	<i>Microtus</i>	52	A/A	[9]
<i>M. mexicanus</i> ^(a)	<i>Aulacomys</i>	48	A/A	[38]
<i>M. gregalis</i>	<i>Stenocranius</i>	36	M/A	[38]
<i>M. duodecimcostatus</i>	<i>Terricola</i>	62	SM/A	[40]
<i>M. majori</i>	<i>Terricola</i>	54	M/A	[9]
<i>M. subterraneus</i>	<i>Terricola</i>	52	M/A	This paper
<i>M. thomasi</i>	<i>Terricola</i>	38-44	SA,A/A,M	[41]

M – metacentric; SM – submetacentric; A – acrocentric; SA – subacrocentric

[†] Subgenera classification based on phylogenetic analysis [42,43] except ^(a) which is classified according to [38].

mosomes were heterochromatic with the X chromosome, being larger than the Y. The absence of synapsis between them was observed in all stages of prophase I of meiosis.

Pairing of sex chromosomes during meiosis was investigated in 26 species, of which 18 were asynapctic (Table 1), including the European pine vole *M. subterraneus* for the first time.

DISCUSSION

Chromosome polymorphisms in *M. subterraneus*, besides the existence of two cytotypes, include sex chromosomes morphology and pairing behavior. A

medium-sized acrocentric Y-chromosome was previously described for pine voles from Serbia [19] and was also found in animals from the Austrian Alps [24], the Ryazan region of Russia [18] and Greece [25]. In contrast, karyotypes of this species with a small Y-chromosome occurred in parts of the Balkan Peninsula [26], the Slovak Tatras, Ukrainian Carpathians and Russia, the Upper Don [26]. Živković et al. [19] analyzed 20 males of *M. subterraneus* from nine localities in the former Yugoslavia. Nineteen of them had karyotypes that were the same as those we identified in animals from Vlasina Rid and Mt. Jastrebac. However, one male had 2n=52 with a small metacentric Y, which the authors considered as a possible aberrant karyotype. Later, Çolak et al. [16] found specimens in the European part of Turkey with 2n=52 and the same small metacentric Y. Morphological variation of the Y chromosome of the 2n=52 cytotype involves the metacentric as well as small and large acrocentric forms, with the metacentric form being the rarest.

In the majority of eutherian male mammals, sex chromosomes display synapsis and crossing over, followed by chiasmata formation. The genus *Microtus* comprises more than sixty species. The karyotypes of about forty of them have been studied, mostly in the subgenus *Microtus* [28]. All examined species of subgenera *Terricola* and *Microtus* have asynapctic sex chromosomes, while all species from subgenera *Alexandromys* are synapctic. In *Terricola*, three species are clearly asynapctic, while separated sex chromosomes dominate in *M. duodecimcostatus*, but different types of pairing are also present [38].

A limited number of attempts has been made to explain the origin of asynapctic sex chromosomes in the genus *Microtus*. However, no satisfactory explanation regarding the mechanisms that enable correct disjunction of sex chromosomes in asynapctic species has been reached. It is more likely that the synapctic condition is the primitive trait and asynapctic the derived one. Besides having synapctic and asynapctic sex chromosomes, they are characterized by different abnormal sex determination systems and polymorphisms in the appearance and structure of sex chromosomes [11]. Thus, it seems that there is an innate property of the genome in this group that allows for the differences in sex determination.

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Author contributions: MR, VJ, TA, IB and BP collected the animals at Vlasina, were engaged in chromosome preparation and preparation of the final version of the manuscript. JB and MV collected the animals at Mt Jastrebac. JB and MR applied the technique of differential chromosome painting. JB performed the chromosome analyses and wrote the manuscript. MV performed the chromosome analyses, assisted with the interpretation of data and supervised the writing of the manuscript.

Conflict of interest disclosure: The authors declare that there are no conflicts of interest.

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